ZooKeys 1129: 21–35 (2022)
doi: 10.3897/zookeys.1129.82981
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

A comparison of gene organisations and phylogenetic relationships of all 22 squamate species listed in South Korea using complete mitochondrial DNA

Daesik Park[1], Il-Hun Kim[2], Il-Kook Park[1], Alejandro Grajal-Puche[3], Jaejin Park[1]

1 *Kangwon National University, Chuncheon, Republic of Korea* **2** *National Marine Biodiversity Institute of Korea, Seochun, Republic of Korea* **3** *Northern Arizona University, Flagstaff, Arizona, USA*

Corresponding author: Jaejin Park (zhqnfth1217@naver.com)

Academic editor: Johannes Penner | Received 2 March 2022 | Accepted 16 September 2022 | Published 10 November 2022

https://zoobank.org/E56CE31A-C614-46D7-9A88-9B4A26C11340

Citation: Park D, Kim I-H, Park I-K, Grajal-Puche A, Park J (2022) A comparison of gene organisations and phylogenetic relationships of all 22 squamate species listed in South Korea using complete mitochondrial DNA. ZooKeys 1129: 21–35. https://doi.org/10.3897/zookeys.1129.82981

Abstract

Studies using complete mitochondrial genome data have the potential to increase our understanding on gene organisations and evolutionary species relationships. In this study, we compared complete mitochondrial genomes between all 22 squamate species listed in South Korea. In addition, we constructed Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic trees using 13 mitochondrial protein-coding genes. The mitochondrial genes for all six species in the suborder Sauria followed the same organisation as the sequenced Testudines (turtle) outgroup. In contrast, 16 snake species in the suborder Serpentes contained some gene organisational variations. For example, all snake species contained a second control region (*CR2*), while three species in the family Viperidae had a translocated *tRNA-Pro* gene region. In addition, the snake species, *Elaphe schrenckii*, carried a *tRNA-Pro* pseudogene. We were also able to identify a translocation of a *tRNA-Asn* gene within the five tRNA (*WANCY* gene region) gene clusters for two true sea snake species in the subfamily Hydrophiinae. Our BI phylogenetic tree was also well fitted against currently known Korean squamate phylogenetic trees, where each family and genus unit forms monophyletic clades and the suborder Sauria is paraphyletic to the suborder Serpentes. Our results may form the basis for future northeast Asian squamate phylogenetic studies.

Keywords

Full mitochondrial genome, Korea, phylogeny, rearrangement, Squamata, *tRNA-Pro*, *WANCY*

Copyright *Daesik Park et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Elucidating comparative gene organisations and phylogenetic relationships between species is essential for improving our understanding of their evolutionary histories and may allow for the successful conservation of endangered taxa (Vane-Wright et al. 1991; Crozier 1992; Boore et al. 1995; Mouquet et al. 2012). Knowledge of genetic organisational variations often give further insights into the evolutionary history of species and could be helpful in developing conservation management plans (Swenson 2019). Additionally, gene organisation data provide reliable phylogenetic information within the evolutionary branches (Boore 1999). Furthermore, defining a phylogenetic species, using mitochondrial and nuclear genes, is often vital to designate if a species is endangered at a national level (An et al. 2010).

The mitochondrial genome is an important model system used to understand genome structure, molecular evolution and phylogenetic relationships amongst vertebrates (Moritz and Brown 1986; Pääbo et al. 1991; Inoue et al. 2003; Krishnan et al. 2004; Jiang et al. 2007; Qian et al. 2018). Specific mitochondrial genes, such as the 12S ribosomal RNA (*12S rRNA*), 16S ribosomal RNA (*16S rRNA*), NADH dehydrogenase subunit 4 (*ND4*), cytochrome c oxidase subunit I (*COI*) and cytochrome b (*Cytb*) have been often used to compare phylogenetic relationships between vertebrates (Malhotra and Thorpe 2004; Rivera et al. 2018; Sidharthan et al. 2021). Although phylogenetic studies using mitochondrial sequences have both advantages and disadvantages (Rubinoff and Holland 2005), using the complete mitochondrial genome may provide higher phylogenetic resolution between species (Inoue et al. 2003; Nardi et al. 2003; Qian et al. 2018). In addition, a comparison of gene organisations, using complete mitochondrial genomes, could give a more precise understanding of the evolutionary histories between species (Boore 1999). To date, a total of 6,781 complete vertebrate mitochondrial genome sequences have been deposited in GenBank (National Center for Biotechnology Information; NCBI), of which 303 are squamates. These 303 sequenced mitochondrial genomes account for approximately 3.8% of the total 7,953 known squamate species (http://www.reptile-database.org/).

Squamates are the most speciose order amongst reptiles, have high ecological and morphological diversity and are found nearly worldwide, with the exception of Antarctica (Greene 1997; Pianka et al. 2003; Vitt and Caldwell 2013). Squamates are divided into two suborders, Sauria and Serpentes. In South Korea, there are six saurian species (five lizards and one gecko species) across four genera and 16 snake species across 10 genera. Together, these squamate species account for 71.0% of all South Korean reptiles (two orders, 11 families, 22 genera and 31 species). In South Korea, there have been several phylogenetic studies mapping reptilian phylogenetic associations. Lee (2010) morphologically demarcated several lizard species, while Lee (2011) combined two *Elaphe schrenckii* subspecies by comparing specific mitochondrial genes and microsatellite alleles. *Rhabdophis tigrinus*, listed in both South Korea and Japan, was recently reclassified as *R. lateralis*, based on mitochondrial genes (Takeuchi et al. 2014). More recently, Kim (2018) applied morphological data in addition to complete

mitochondrial genome sequences to classify sea snake species found in Korean waters. Only one large-scale reptilian phylogenetic study has phylogenetically distinguished between Korean serpent species, based on both morphological and isozyme data (Paik 1982). Thus far, there have been no phylogenetic reconstructions using gene organisations amongst Korean squamates using complete mitochondrial genomes.

Recent phylogenetic molecular advances have made it more possible to compare complete mitochondrial genome relationships between species (Lee et al. 2021; Zhang et al. 2021; Vanerelli et al. 2022). These molecular advances make it a prime occasion to update our understanding of the phylogenetic relationships amongst squamates, specifically in South Korea. In this study, we compared mitochondrial gene organisations and constructed three types of phylogenetic trees amongst all 22 squamate species listed in South Korea, using complete mitochondrial sequence data. These results have the potential to impact future squamate phylogenetic studies in northeast Asia.

Materials and methods

Complete mitochondrial DNA (mtDNA) sequences, for all 22 squamate species listed in South Korea, were downloaded from GenBank (NCBI). We tried to obtain mtDNA sequence data from individuals, captured within South Korea. When it was not possible, we used mtDNA data from specimens collected in China, Japan or Russia (Table 1) that were within the distribution range of the same species listed in South Korea (Takeuchi et al. 2014; Lee et al. 2022). The downloaded, foreign-originated sequences from GenBank showed high similarity with the preliminary BLAST analysis using partial 16S ribosomal RNA (*16S rRNA*), cytochrome oxidase subunit I (*COI*) or cytochrome b (*Cytb*) sequences of South Korean specimens (Jeong et al. 2013). For *Hydrophis cyanocinctus* and *H. melanocephalus*, we used the data from individuals caught at locations nearest to South Korea, as there were no data available from South Korean specimens. After confirming complete mitochondrial genes for each species, we arranged and compared the mitochondrial genes between species, based on transfer RNA (tRNA) positions and ribosomal RNA (rRNA) and protein-coding gene (PCG) positions, which were determined using tRNAscan-SE 2.0 (Chan and Lowe 2019) and MITOS Webserver (Bernt et al. 2013), respectively. We excluded *H. cyanocinctus* from the analysis due to the uncertainty of the nucleotide sequence in non-protein-coding regions, such as rRNA, tRNA, O_L and CR within a cluster of five tRNA genes (*WANCY* gene region).

To construct the phylogenetic tree of all 22 squamate species listed in South Korea, we used 13 mitochondrial PCGs (Fenn et al. 2008; Douglas and Gower 2010; He et al. 2010). The sequence of each PCG was extracted and aligned using MUSCLE, with a maximum of 20 iterations (Edgar 2004). Aligned sequence genes were concatenated into one sequence using Geneious v.9.1.8 (Kearse et al. 2012). We used two sea turtle species, *Caretta caretta* and *Chelonia mydas*, as outgroups for our constructed phylogenetic tree. We used PAUP 4.0a168 (Swofford 2001) to create the Maximum Parsimony (MP) phylogenetic tree. To increase the reliability of the MP phylogenetic tree, we performed

Table 1. All 22 analysed squamate species listed in South Korea and their sampling locations analysed in this study. Original sample location, GenBank accession number and mt-genome base pair length are provided.

Suborder	Family	Species	Location	GenBank No.	Length (bp)
Sauria	Gekkonidae	*Gekko japonicus*	S. Korea	KR996131	16,544
	Scincidae	*Scincella vandenburghi*	S. Korea	KU646826	17,103
		Scincella huanrenensis	S. Korea	KU507306	17,212
	Lacertidae	*Takydromus amurensis*	China	KU641018	17,333
		Takydromus wolteri	China	JX181764	18,236
		Eremias argus	S. Korea	JQ086345	18,521
Serpentes	Colubridae	*Elaphe dione*	Russia	MH460961	17,172
		Elaphe schrenckii	China	KP888955	17,165
		Oocatochus rufodorsatus	China	KC990020	17,159
		Rhabdophis lateralis	China	KU641019	17,415
		Hebius vibakari	China	KP684155	17,259
		Lycodon rufozonatus	China	KJ179950	17,188
		Orientocoluber spinalis	S. Korea	MT304473	17,196
		Sibynophis chinensis	S. Korea	KF360246	17,163
	Viperidae	*Gloydius ussuriensis*	China	KP262412	17,208
		Gloydius brevicaudus	China	EU913477	17,227
		Gloydius saxatilis	S. Korea	MW143075	17,223
	Elapidae	*Hydrophis cyanocinctus*	China	MK953550	17,750
		Hydrophis melanocephalus	Japan	MK775532	17,182
		Hydrophis platurus	S. Korea	MK775530	18,101
		Laticauda laticaudata	S. Korea	KY496323	17,209
		Laticauda semifasciata	S. Korea	KY496325	17,170
Cryptodira	Cheloniidae	*Chelonia mydas*	Cyprus	JX454990	16,495
		Caretta caretta	USA	JX454983	16,454

1,000 bootstrap iterations. For estimating the MP tree, we applied a heuristic search using tree bisection and reconnection (TBR) and branch swapping approaches. We used RAxML v.7.2.8 (Stamatakis 2006) to create the Maximum Likelihood (ML) phylogenetic tree. To select the best nucleotide model for the ML analysis, we used jModelTest 2.1.10 (Darriba et al. 2012) and selected the GTR GAMMA I model through AIC calculations. The rapid bootstrapping value was calculated while selecting the best-scoring tree from 100 ML trees. To construct the Bayesian Inference (BI) phylogenetic tree, we used MrBayes v.3.2.6 (Huelsenbeck and Ronquist 2001). For the BI phylogenetic tree, each substitution model and rate variation was set to GTR and gamma, respectively. For the Markov Chain Monte Carlo (MCMC) analysis of the BI tree, the chain length was set to 1,000,000, while the subsampling frequency was set to 100. The MCMC heated chain was also set to 4, the heated chain temperature was set to 0.2 and the burn-in cut-off was at 50,000.

Results

All six species within the suborder Sauria matched the mitochondrial organisation of the sea turtle outgroup (Fig. 1). In contrast, all 16 snake species in the suborder Serpentes had a second control region (*CR2*) between the NADH dehydrogenase subunit 1 (*ND1*) and the NADH dehydrogenase subunit 2 (*ND2*). These 16 snake species also

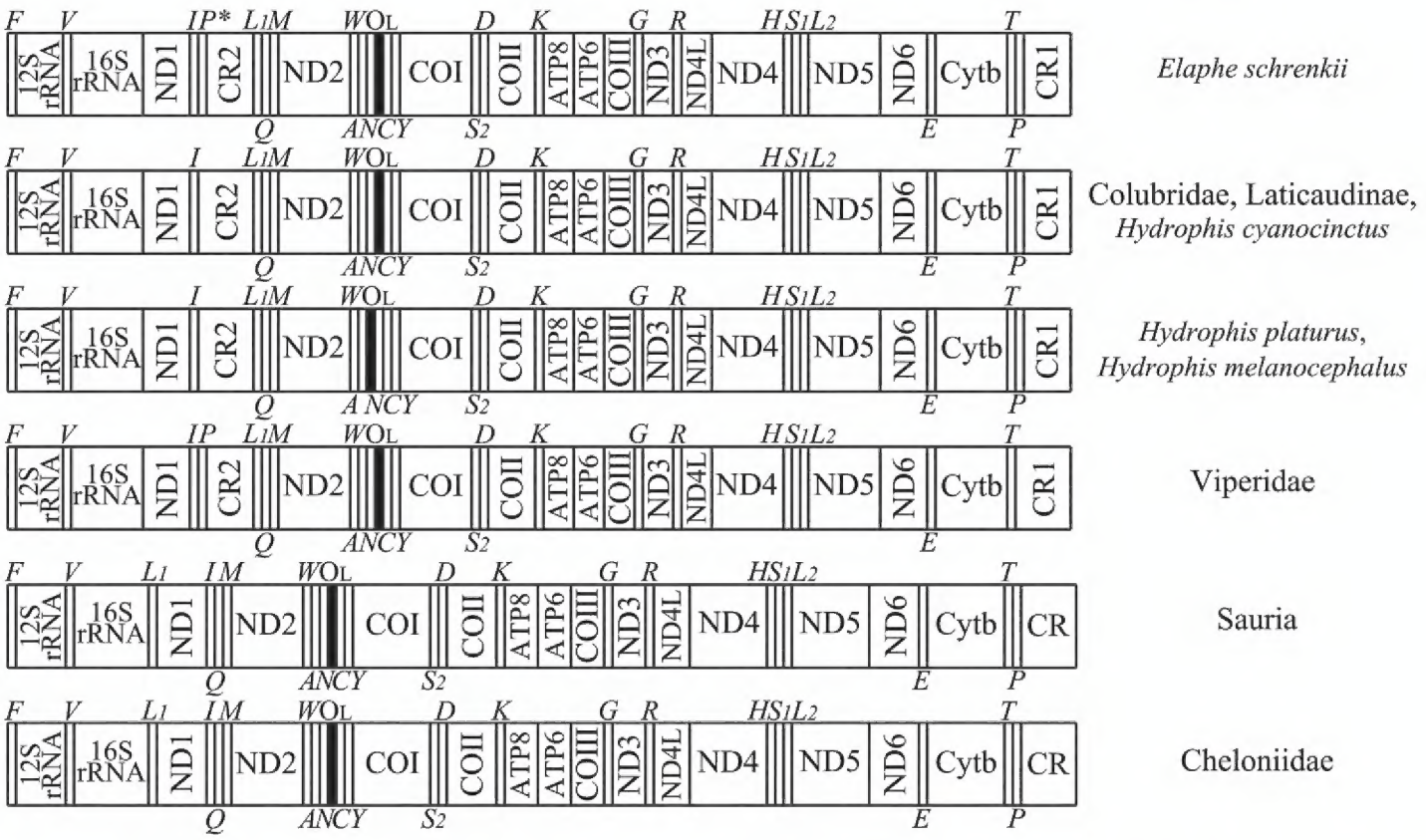

Figure 1. Comparison of the mitochondrial gene organisation for all 22 squamate species listed in South Korea. Two sea turtle species (*Caretta caretta* and *Chelonia mydas*), served as outgroups. *L1*, *L2*, *S1*, *S2*. *P** represent the following genes: *tRNA-Leu* (UUR), *tRNA-Leu* (CUN), *tRNA-Ser* (AGY), *tRNA-Ser* (UCN) and a pseudo *tRNA-Pro* gene, respectively. tRNA genes are abbreviated by the corresponding one-letter amino acid codes, such as *F, V, I, M* etc.

had translocated *tRNA-Leu* (UUA) genes between *tRNA-Ile* and *tRNA-Gln*. In addition, three species in the family Viperidae had a translocated *tRNA-Pro* gene. The *tRNA-Pro* gene was originally between *tRNA-Thr* and *CR1* genes, but was translocated in between the *tRNA-Ile* and *CR2* genes. Moreover, the three viperid species lacked any *tRNA-Pro* pseudogenes observed in the other serpent species. In *H. platurus* and *H. melanocephalus*, the *tRNA-Asn* gene, originally located between *tRNA-Ala* and the light strand replication origin (O_L) in other snakes, had also been translocated between the O_L region and the *tRNA-Cys* gene within the WANCY cluster region. Finally, a pseudo-*tRNA-Pro* gene was discovered between the *tRNA-Ile* and *CR2* genes in *Elaphe schrenckii*.

The aligned sequence data for the 13 mitochondrial protein-coding genes were concatenated to 11,581 bp. The suborders Sauria and Serpentes each formed monophyletic clades in both MP and ML phylogenetic trees (Fig. 2). In contrast, all six species within the suborder Sauria were paraphyletic to the suborder Serpentes in the BI tree (Fig. 3). Branching patterns were the same between Viperidae and Elapidae families amongst our three constructed phylogenetic trees. Specifically, two species in the family Viperidae, *Gloydius ussuriensis* and *G. brevicaudus*, were more closely related, while *G. saxatilis* formed a sister clade. Within the genus *Hydrophis*, *H. cyanocinctus* and *H. melanocephalus* clustered more closely together than *H. platurus*. For the family Colubridae, species in the BI tree were paraphyletic, which differed from our constructed ML and MP trees. In the BI tree, *Gekko japonicus* was basal to all other saurian species.

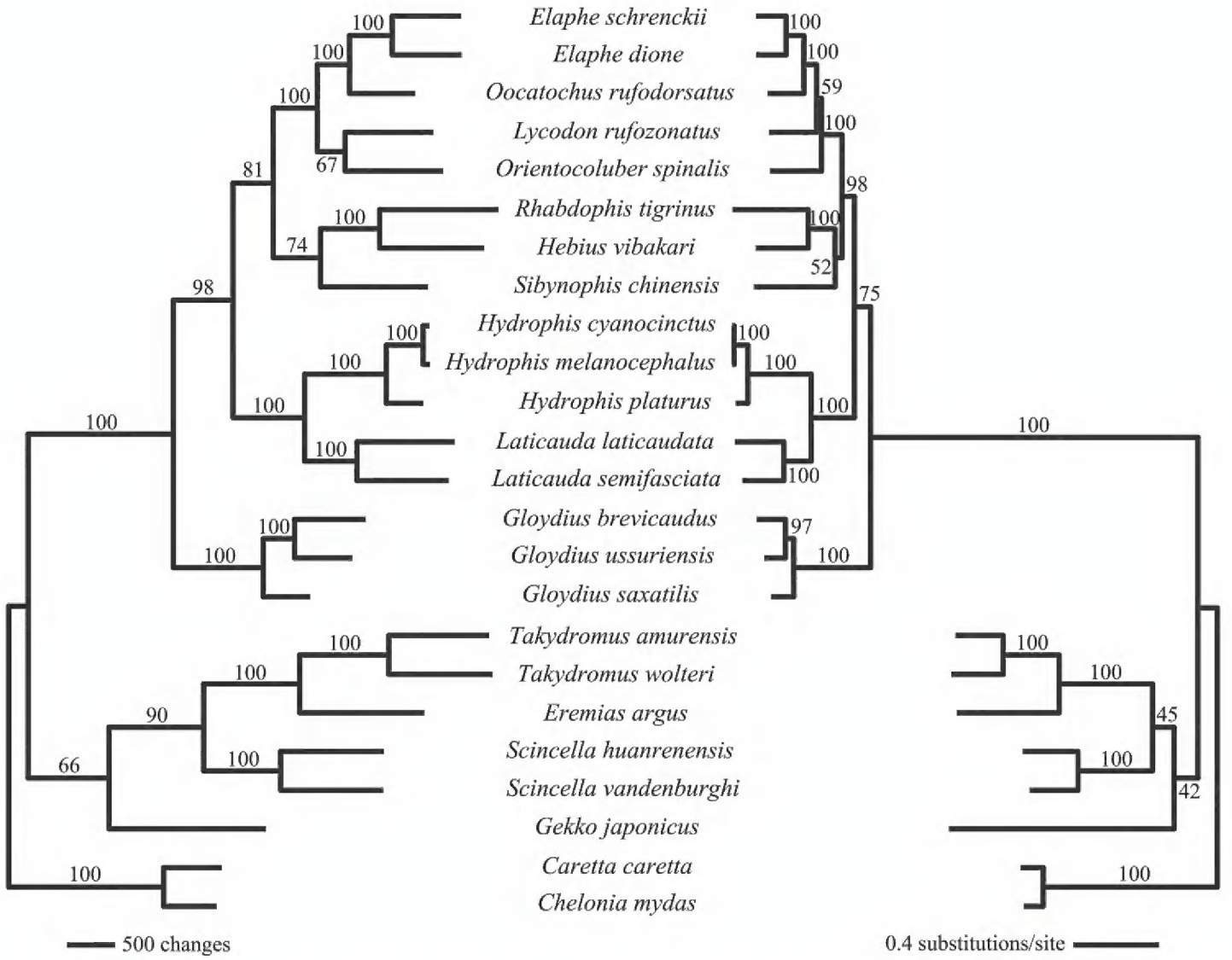

Figure 2. Maximum Parsimony (MP) tree (left) and Maximum Likelihood (ML) tree (right) for all 22 squamates listed in South Korea. Both MP and ML trees are based on 13 mitochondrial protein-coding genes. *Caretta caretta* and *Chelonia mydas* are outgroup species. Bootstrap values are denoted on each tree branch.

Discussion

We compared gene organisations and elucidated phylogenetic relationships, using complete mitochondrial genomes, for all 22 squamate species listed in South Korea. Using this complete mtDNA approach, we were able to uncover unique evolutionary insights amongst South Korean squamates.

Relative to the six saurian species, we discovered that all 16 snake species in the suborder Serpentes had an additional control region (*CR2*) and a translocated *tRNA-Leu* (UUR) gene. These genetic characteristics have been documented in many other snake species, except for the infraorder Scolecophidia (Douglas et al. 2006; Jiang et al. 2007). The *CR2* was located between *ND1* and *ND2* genes and may be an example of a non-independent replication event (Dover 1982; Kumazawa et al. 1998; Dong and Kumazawa 2005). The presence of two control regions may increase the rate of mitochondrial gene replication (Jiang et al. 2007). Duplication of the *CR* is not novel amongst vertebrates and has been identified in fishes (Lee et al. 2001; Inoue et al. 2003), frogs (Sano et al. 2005; Huang et al. 2019), lizards (Kumazawa and Endo 2004; Amer and Kumazawa

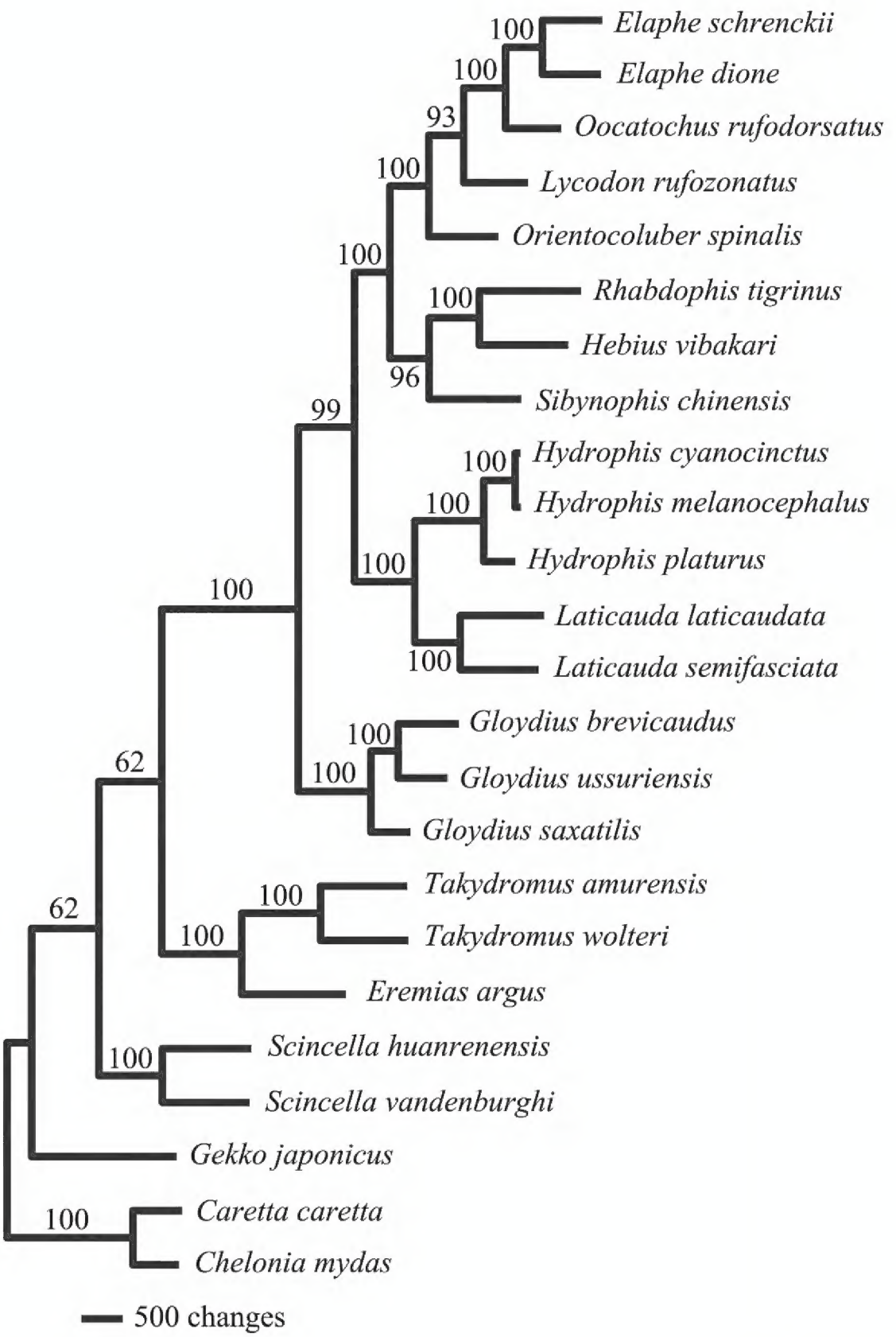

Figure 3. The constructed Bayesian Inference (BI) tree for all 22 squamate species listed in South Korea, based on 13 mitochondrial protein-coding genes. Two cheloniid species (*Caretta caretta* and *Chelonia mydas*) were used as outgroups. Bayesian posterior probabilities are denoted on each tree branch.

2005) and birds (Eberhard et al. 2001; Sammler et al. 2011). The translocation of *tRNA-Leu* (UUR) gene, identified in this study, is considered an ancestral trait of the infraorder Alethinophidia (Dong and Kumazawa 2005). In vertebrates, the *tRNA-Leu* gene has been shown to alter the transcribed ratios between the rRNA and protein-coding gene as terminators for heavy-stranded transcripts (Fernández-Silva et al. 2003). The presence

of the *CR2* and the translocation of the *tRNA-Leu* gene might also prevent a thermodynamic depression of transcriptional enzymes in snakes (Jiang et al. 2007).

All three *Gloydius* species, listed in South Korea, had translocated *tRNA-Pro* gene regions, which is consistent with other previously reported Viperidae species, such as *Agkistrodon piscivorus* (Jiang et al. 2007), *Causus defilippi* (Castoe et al. 2009), *Bothrops pubescens* (GenBank No. MG182598) and *Vipera berus* (Gao et al. 2018). In contrast, we did not detect any half-sized *tRNA-Pro* pseudogenes from the three analysed *Gloydius* species, although the half-sized pseudogenes have been previously reported in a closely-related species, *G. strauchi* (GenBank No. MF523224). The half-sized tRNA-Pro pseudogene, adjacent to the 5' end of *CR1*, has also been reported in *Ovophis okinavensis* (Kumazawa et al. 1996) and in several other crotaline species, such as *Protobothrops kaulbacki* (Kang et al. 2017), *Trimeresurus albolabris* (Song et al. 2015) and *T. sichuanensis* (Zhu et al. 2016). The half-sized *tRNA-Pro* pseudogene is considered a shared ancestral trait amongst crotaline species and may have been lost and subsequently recovered independently between species (Wüster et al. 2008).

In *H. platurus* and *H. melanocephalus*, the *tRNA-Asn* gene was translocated between the O_L and *tRNA-Cys* genes. The *tRNA-Asn* gene is usually located between *tRNA-Ala* and O_L in the WANCY region in other snakes and vertebrates (Boore 2000; Qian et al. 2018; Fig. 4). To our knowledge, this is the first finding of a *tRNA-Asn* translocation in the infraorder Alethinophidia. Mitochondrial genetic rearrangements are often found near control or WANCY regions in serpents (Qian et al. 2018). Tandem duplication random loss (TDRL) models often explain that some gene rearrangements may occur due to random paralog deletions (Moritz et al. 1987; Boore 2000; Xiaokaiti et al. 2022). These random paralog deletions may explain our results, as paralog deletions have caused gene rearrangements in other vertebrates including lizards, marsupials, birds and fishes (Moritz and Brown 1986, 1987; Moritz et al. 1987; Pääbo et al. 1991; Boore 2000; Inoue et al. 2003). We were also able to identify a novel gene rearrangement in sea snakes found off the coasts of South Korea. To our knowledge, this gene rearrangement has not been detected in other elapid species, including cobras (Yan et al. 2008; Castoe et al. 2009; Singchat et al. 2019) and sea kraits (Kim et al. 2018).

The existence of the intergenic spacers, which are non-coding regions between genes, is evidence of certain genetic deletions (San Mauro et al. 2006; Xiaokaiti et al. 2022). The gene rearrangement observed in the true sea snake species may be the result of a duplication of the *WANCY* genes and subsequent deletions of certain parts of other genes (Fig. 4A). We were also able to corroborate the occurrence of similar gene rearrangements in two other foreign sea snake species, *Emydocephalus ijimae* and *H. curtus*, for which complete mt-genome were available (GenBank MK775531, MT712129; Yi et al. 2019; Zhang and Yan 2020) (Fig. 4B, C). This WANCY gene rearrangement was not present in the four sequenced terrestrial cobra species (*Bungarus fasciatus*, *Micrurus fulvius*, *Naja naja* and *Sinomicrurus macclellandi*; GenBank No. EU579523, GU045453, DQ343648, MT547176; Yan et al. 2008; Castoe et al. 2009; Yao et al. 2020). Uploading and comparing additional mt-genome data for closely-related Australian terrestrial cobras and sea snakes may

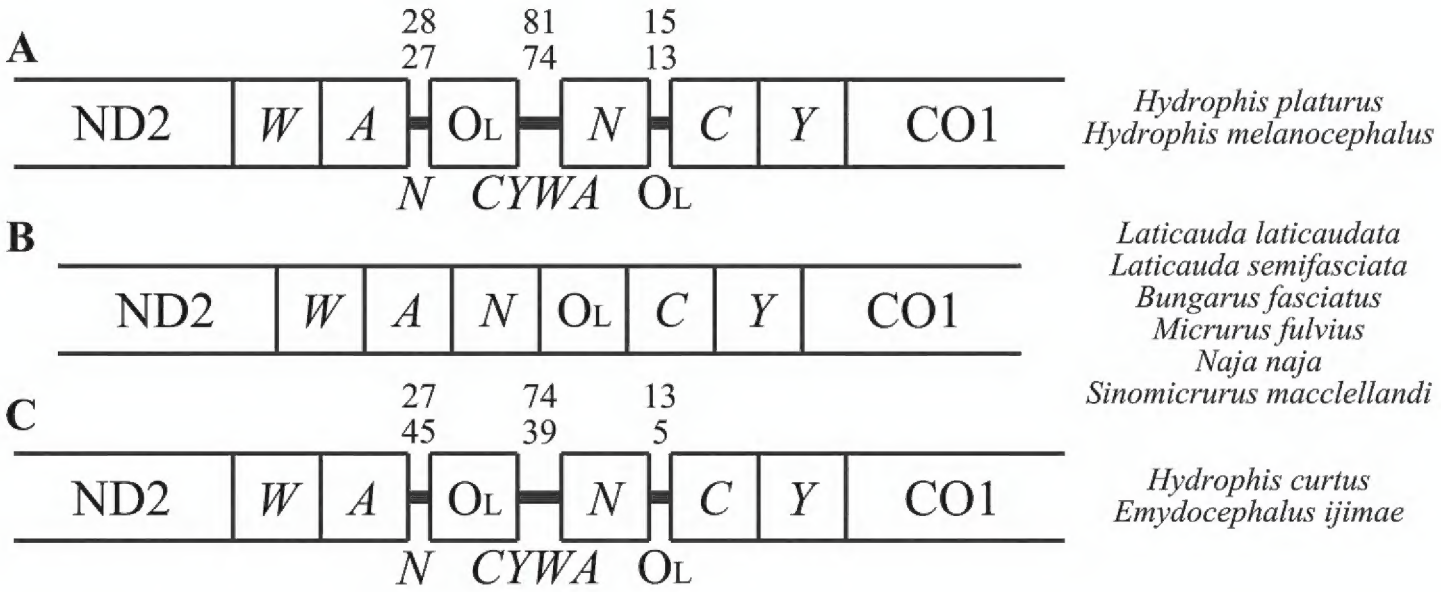

Figure 4. Intergenic spacers located in the cluster of five tRNA genes (*WANCY* gene region) of two sea snake species (*Hydrophis platurus* and *H. melanocephalus*) found off South Korean coastal waters **A** intergenic spacers located in the *WANCY* gene region of two sea krait species (*Laticauda laticaudata* and *L. semifasciata*), four terrestrial cobras (*Bungarus fasciatus*, *Micrurus fulvius*, *Naja naja* and *Sinomicrurus macclellandi*) **B** and two true sea snake species downloaded from GenBank (*H. curtus* and *Emydocephalus ijimae*) **C** intergenic spacer lengths amongst Hydrophiinae species are above the intergenic spacer in the same order as the species are listed. The hypothesised tRNA genes lost due to random deletions are written below the intergenic spacers. The tRNA genes are abbreviated by the corresponding one-letter amino acid codes, such as *W*, *A*, *N* etc.

give further insights into elapid speciation and possibly adaptation from terrestrial to oceanic ecosystems.

Our constructed BI phylogenetic tree was well fitted amongst known Korean squamate phylogenies. In our BI tree, the suborder Sauria was paraphyletic to the superfamily Lacertoidea and was sister to the suborder Serpentes. In addition, the family Gekkonidae formed an independent branch at the most basal position, which is consistent with previous studies (Townsend et al. 2004; Vidal and Hedges 2005; Hugall et al. 2007; Wiens et al. 2012; Pyron et al. 2013; Reeder et al. 2015). Our constructed BI tree also corroborated a recent phylogenetic investigation which used both morphological characters and specific mitochondrial and nuclear genes of 200 squamate species (Reeder et al. 2015). Due to the parallels between our constructed BI tree and the aforementioned study, we hypothesise that South Korean squamates most likely speciated in this manner.

In this study, we used complete mitochondrial sequenced genomes to determine mitochondrial gene organisations and phylogenetic relationships amongst all 22 squamate species listed in South Korea. The analysed species appear to have several unique mitochondrial rearrangements, including family and order-specific gene duplications and translocations. Overall, our constructed BI phylogenetic tree was well fitted amongst Korean squamates and is consistent with other phylogenetic studies which utilised specific mt-gene sequences. These results may form the basis of future phylogenetic investigations, clarifying northeast Asian squamate speciation.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2020R1I1A3051885) and National Marine Biodiversity Institute of Korea (2022M00300).

References

Amer SAM, Kumazawa Y (2005) Mitochondrial genome of *Pogona vitticepes* (Reptilia; Agamidae): Control region duplication and the origin of Australasian agamids. Gene 346: 249–256. https://doi.org/10.1016/j.gene.2004.11.014

An JH, Park DS, Lee JH, Kim KS, Lee H, Min MS (2010) No genetic differentiation of *Elaphe schrenckii* subspecies in Korea based on 9 microsatellite loci. Animal Systematics, Evolution and Diversity 26(1): 15–19. https://doi.org/10.5635/KJSZ.2010.26.1.015

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF (2013) MITOS: Improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69(2): 313–319. https://doi.org/10.1016/j.ympev.2012.08.023

Boore JL (1999) Animal mitochondrial genomes. Nucleic acids research 27(8): 1767–1780. https://doi.org/10.1093/nar/27.8.1767

Boore JL (2000) The duplication/random loss model for gene rearrangement exemplified by mitochondrial genomes of deuterostome animals. In: Sankoff D, Nadezu JH (Eds) Comparative genomics. Springer Press, Dordrecht, 133–147. https://doi.org/10.1007/978-94-011-4309-7_13

Boore JL, Collins TM, Stanton D, Daehler LL, Brown WM (1995) Deducing the pattern of arthropod phylogeny from mitochondrial DNA rearrangements. Nature 376(6536): 163–165. https://doi.org/10.1038/376163a0

Castoe TA, Gu W, De Koning APJ, Daza JM, Jiang ZJ, Parkinson CL, Pollock DD (2009) Dynamic nucleotide mutation gradients and control region usage in squamate reptile mitochondrial genomes. Cytogenetic and Genome Research 127(2–4): 112–127. https://doi.org/10.1159/000295342

Chan PP, Lowe TM (2019) tRNAscan-SE: Searching for tRNA genes in genomic sequences. In: Kollmar M (Eds) Gene Prediction, Springer Press, New York, 1–14. https://doi.org/10.1007/978-1-4939-9173-0_1

Crozier RH (1992) Genetic diversity and the agony of choice. Biological Conservation 61(1): 11–15. https://doi.org/10.1016/0006-3207(92)91202-4

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109

Dong S, Kumazawa Y (2005) Complete mitochondrial DNA sequences of six snakes: Phylogenetic relationships and molecular evolution of genomic features. Journal of Molecular Evolution 61(1): 12–22. https://doi.org/10.1007/s00239-004-0190-9

Douglas DA, Gower DJ (2010) Snake mitochondrial genomes: Phylogenetic relationships and implications of extended taxon sampling for interpretations of mitogenomic evolution. BMC Genomics 11(1): 1–16. https://doi.org/10.1186/1471-2164-11-14

Douglas DA, Janke A, Arnason U (2006) A mitogenomic study on the phylogenetic position of snakes. Zoologica Scripta 35(6): 545–558. https://doi.org/10.1111/j.1463-6409.2006.00257.x

Dover G (1982) Molecular drive: A cohesive mode of species evolution. Nature 299(5879): 111–117. https://doi.org/10.1038/299111a0

Eberhard JR, Wright TF, Bermingham E (2001) Duplication and concerted evolution of the mitochondrial control region in the parrot genus *Amazona*. Molecular Biology and Evolution 18(7): 1330–1342. https://doi.org/10.1093/oxfordjournals.molbev.a003917

Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Fenn JD, Song H, Cameron SL, Whiting MF (2008) A preliminary mitochondrial genome phylogeny of Orthoptera (Insecta) and approaches to maximizing phylogenetic signal found within mitochondrial genome data. Molecular Phylogenetics and Evolution 49(1): 59–68. https://doi.org/10.1016/j.ympev.2008.07.004

Fernández-Silva P, Enriquez JA, Montoya J (2003) Replication and transcription of mammalian mitochondrial DNA. Experimental Physiology 88(1): 41–56. https://doi.org/10.1113/eph8802514

Gao D, Liu C, Hao S (2018) Characterization of the complete mitochondrial genome of the European adder (*Vipera berus*) using Illumina sequencing technology. Conservation Genetics Resources 10(4): 793–796. https://doi.org/10.1007/s12686-017-0932-9

Greene HW (1997) Snakes: The evolution of mystery in nature. University of California Press, Berkeley, 477 pp. https://doi.org/10.1525/9780520935433

He M, Feng J, Zhao E (2010) The complete mitochondrial genome of the Sichuan hot-spring keelback (*Thermophis zhaoermii*; Serpentes: Colubridae) and a mitogenomic phylogeny of the snakes. Mitochondrial DNA 21(1): 8–18. https://doi.org/10.3109/19401730903505867

Huang A, Li H, Luo H, Ni Q, Yao Y, Xu H, Zeng B, Li Y, Wei Z, Zhang M (2019) The complete mitochondrial genome of the tree frog, *Polypedates braueri* (Anura, Rhacophoridae). Mitochondrial DNA Part B 4(1): 1739–1740. https://doi.org/10.1080/23802359.2019.1607594

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Hugall AF, Foster R, Lee MS (2007) Calibration choice, rate smoothing, and the pattern of tetrapod diversification according to the long nuclear gene RAG-1. Systematic Biology 56(4): 543–563. https://doi.org/10.1080/10635150701477825

Inoue JG, Miya M, Tsukamoto K, Nishida M (2003) Evolution of the deep-sea gulper eel mitochondrial genomes: Large-scale gene rearrangements originated within the eels. Molecular Biology and Evolution 20(11): 1917–1924. https://doi.org/10.1093/molbev/msg206

Jeong TJ, Jun J, Han S, Kim HT, Oh K, Kwak M (2013) DNA barcode reference data for the Korean herpetofauna and their applications. Molecular Ecology Resources 13(6): 1019–1032. https://doi.org/10.1111/1755-0998.12055

Jiang ZJ, Castoe TA, Austin CC, Burbrink FT, Herron MD, McGuire JA, Parkinson CL, Pollock DD (2007) Comparative mitochondrial genomics of snakes: Extraordinary substitution rate dynamics and functionality of the duplicate control region. BMC Evolutionary Biology 7(1): 1–14. https://doi.org/10.1186/1471-2148-7-123

Kang X, Zhang Y, Qian L, Sun P, Wang C, Fang K, Pan T, Zhang B, Rao D, Wang H (2017) The complete mitochondrial genome of *Protobothrops kaulbacki* (Squamata: Viperidae). Mitochondrial DNA Part B 2(1): 201–202. https://doi.org/10.1080/23802359.2017.1307703

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kim IH (2018) Taxonomy and molecular phylogeny of sea snakes (Hydrophinae and Laticaudinae) in Korea. PhD Thesis, Kangwon National University, Chuncheon, South Korea.

Kim IH, Park J, Suk HY, Bae HG, Min MS, Tsai TS, Park D (2018) Phylogenetic relationships of three representative sea krait species (Genus *Laticauda*; Elapidae; Serpentes) based on 13 mitochondrial genes. Mitochondrial DNA Part A 29(5): 772–777. https://doi.org/10.1080/24701394.2017.1357710

Krishnan NM, Raina SZ, Pollock DD (2004) Analysis of among-site variation in substitution patterns. Biological Procedures Online 6(1): 180–188. https://doi.org/10.1251/bpo88

Kumazawa Y, Endo H (2004) Mitochondrial genome of the Komodo dragon: Efficient sequencing method with reptile-oriented primers and novel gene rearrangements. DNA Research 11(2): 115–125. https://doi.org/10.1093/dnares/11.2.115

Kumazawa Y, Ota H, Nishida M, Ozawa T (1996) Gene rearrangements in snake mitochondrial genomes: Highly concerted evolution of control-region-like sequences duplicated and inserted into a tRNA gene cluster. Molecular Biology and Evolution 13(9): 1242–1254. https://doi.org/10.1093/oxfordjournals.molbev.a025690

Kumazawa Y, Ota H, Nishida M, Ozawa T (1998) The complete nucleotide sequence of a snake (*Dinodon semicarinatus*) mitochondrial genome with two identical control regions. Genetics 150(1): 313–329. https://doi.org/10.1093/genetics/150.1.313

Lee SC (2010) Systematic and ecological studies of the Suborder Sauria (Reptilia, Squamata) in Korea. PhD Thesis, University of Incheon, Incheon, South Korea.

Lee JH (2011) Taxonomic status, habitat use and suitability modeling of the Amur ratsnake (*Elaphe schrenckii*). PhD Thesis, Kangwon National University, Chuncheon, South Korea.

Lee JS, Miya M, Lee YS, Kim CG, Park EH, Aoki Y, Nishida M (2001) The complete DNA sequence of the mitochondrial genome of the self-fertilizing fish *Rivulus marmoratus* (Cyprinodontiformes, Rivulidae) and the first description of duplication of a control region in fish. Gene 280(1–2): 1–7. https://doi.org/10.1016/S0378-1119(01)00765-X

Lee YS, Do MS, Jeon HS, Lee SC, Jung JH, Suh JH, An J (2021) Complete mitochondrial genome of *Gloydius saxatilis* (Viperidae: Crotalinae) from Korea. Mitochondrial DNA Part B 6(2): 645–647. https://doi.org/10.1080/23802359.2021.1878957

Lee YS, Do MS, Kim W, Jeon HS, Lee SC, Jung JH, An J (2022) Phylogenetic relationships between three Korean pit viper *Gloydius* (Serpentes: Crotalinae) species using mitochondrial DNA genes. Genes & Genomics 44(5): 517–526. https://doi.org/10.1007/s13258-022-01222-3

Malhotra A, Thorpe RS (2004) A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pitvipers (*Trimeresurus* and *Ovophis*). Molecular Phylogenetics and Evolution 32(1): 83–100. https://doi.org/10.1016/j.ympev.2004.02.008

Moritz C, Brown WM (1986) Tandem duplication of D-loop and ribosomal RNA sequences in lizard mitochondrial DNA. Science 233(4771): 1425–1427. https://doi.org/10.1126/science.3018925

Moritz C, Brown WM (1987) Tandem duplications in animal mitochondrial DNAs: Variation in incidence and gene content among lizards. Proceedings of the National Academy of Sciences of the United States of America 84(20): 7183–7187. https://doi.org/10.1073/pnas.84.20.7183

Moritz C, Dowling TE, Brown WM (1987) Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. Annual Review of Ecology and Systematics 18(1): 269–292. https://doi.org/10.1146/annurev.es.18.110187.001413

Mouquet N, Devictor V, Meynard CN, Munoz F, Bersier L, Chave J, Couteron P, Dalecky A, Fontaine C, Gravel D, Hardy OJ, Jabot F, Lavergne S, Leibold M, Mouillot D, Münkemüller T, Pavoine S, Prinzing A, Rodrigues ASL, Rohr RP, Thébault E, Thuiller W (2012) Ecophylogenetics: Advances and perspectives. Biological Reviews of the Cambridge Philosophical Society 87(4): 769–785. https://doi.org/10.1111/j.1469-185X.2012.00224.x

Nardi F, Spinsanti G, Boore JL, Carapelli A, Dallai R, Frati F (2003) Hexapod origins: Monophyletic or paraphyletic? Science 299(5614): 1887–1889. https://doi.org/10.1126/science.1078607

Pääbo S, Thomas WK, Whitfield KM, Kumazawa Y, Wilson AC (1991) Rearrangements of mitochondrial transfer RNA genes in marsupials. Journal of Molecular Evolution 33(5): 426–430. https://doi.org/10.1007/BF02103134

Paik NK (1982) Systematic studies on the Suborder Serpentes (Reptilia) in Korea. PhD Thesis, Sungkyunkwan University, Seoul, South Korea.

Pianka ER, Pianka ER, Vitt LJ (2003) Lizards: Windows to the evolution of diversity University of California Press, Berkeley, 246 pp. https://doi.org/10.1525/california/9780520234017.001.0001

Pyron RA, Burbrink FT, Wiens JJ (2013) A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evolutionary Biology 13(1): 1–54. https://doi.org/10.1186/1471-2148-13-93

Qian L, Wang H, Yan J, Pan T, Jiang S, Rao D, Zhang B (2018) Multiple independent structural dynamic events in the evolution of snake mitochondrial genomes. BMC Genomics 19(1): 1–11. https://doi.org/10.1186/s12864-018-4717-7

Reeder TW, Townsend TM, Mulcahy DG, Noonan BP, Wood Jr PL, Sites Jr JW, Wiens JJ (2015) Integrated analyses resolve conflicts over squamate reptile phylogeny and reveal unexpected placements for fossil taxa. PLoS ONE 10(3): e0118199. https://doi.org/10.1371/journal.pone.0118199

Rivera PC, González-Ittig RE, Robainas Barcia A, Trimarchi LI, Levis S, Calderón GE, Gardenal CN (2018) Molecular phylogenetics and environmental niche modeling reveal a cryptic species in the *Oligoryzomys flavescens* complex (Rodentia, Cricetidae). Journal of Mammalogy 99(2): 363–376. https://doi.org/10.1093/jmammal/gyx186

Rubinoff D, Holland BS (2005) Between two extremes: Mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Systematic Biology 54(6): 952–961. https://doi.org/10.1080/10635150500234674

Sammler S, Bleidorn C, Tiedemann R (2011) Full mitochondrial genome sequences of two endemic *Philippine hornbill* species (Aves: Bucerotidae) provide evidence for pervasive mitochondrial DNA recombination. BMC Genomics 12(1): 1–10. https://doi.org/10.1186/1471-2164-12-35

San Mauro D, Gower DJ, Zardoya R, Wilkinson M (2006) A hotspot of gene order rearrangement by tandem duplication and random loss in the vertebrate mitochondrial genome. Molecular Biology and Evolution 23(1): 227–234. https://doi.org/10.1093/molbev/msj025

Sano N, Kurabayashi A, Fujii T, Yonekawa H, Sumida M (2005) Complete nucleotide sequence of the mitochondrial genome of Schlegel's tree frog *Rhacophorus schlegelii* (family Rhacophoridae): Duplicated control regions and gene rearrangements. Genes & Genetic Systems 80(3): 213–224. https://doi.org/10.1266/ggs.80.213

Sidharthan A, Raghavan R, Anoop V, Keskar A, Dahanukar N (2021) Phylogenetic position and relationships of mountain loaches (Teleostei: Balitoridae) of the Western Ghats as revealed by CO1 sequences. Zootaxa 4926(1): 79–92. https://doi.org/10.11646/zootaxa.4926.1.5

Singchat W, Areesirisuk P, Sillapaprayoon S, Muangmai N, Baicharoen S, Suntrarachun S, Chanhome L, Peyachoknagul S, Srikulnath K (2019) Complete mitochondrial genome of Siamese cobra (*Naja kaouthia*) determined using next-generation sequencing. Mitochondrial DNA Part B 4(1): 577–578. https://doi.org/10.1080/23802359.2018.1558123

Song T, Zhang C, Zhang L, Huang X, Hu C, Xue C, Zhang B (2015) Complete mitochondrial genome of *Trimeresurus albolabris* (Squamata: Viperidae: Crotalinae). Mitochondrial DNA 26(2): 291–292. https://doi.org/10.3109/19401736.2013.825780

Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22(21): 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Swenson NG (2019) Phylogenetic ecology: A history, critique, and remodeling. University of Chicago Press, Chicago, 240 pp. https://doi.org/10.7208/chicago/9780226671642.001.0001

Swofford DL (2001) PAUP*. Phylogenetic analysis using parsimony (*and Other Methods). 4.0b10 edn. Sinauer Associates, Inc., Sunderland.

Takeuchi H, Zhu GX, Ding L, Tang Y, Ota H, Mori A, Oh HS, Hikida T (2014) Taxonomic validity and phylogeography of the East Eurasian natricine snake, *Rhabdophis lateralis* (Berthold, 1859)(Serpentes: Colubridae), as inferred from mitochondrial DNA sequence data. Current Herpetology 33(2): 148–153. https://doi.org/10.5358/hsj.33.148

Townsend TM, Larson A, Louis E, Macey JR (2004) Molecular phylogenetics of Squamata: The position of snakes, amphisbaenians, and dibamids, and the root of the squamate tree. Systematic Biology 53(5): 735–757. https://doi.org/10.1080/10635150490522340

Vane-Wright RI, Humphries CJ, Williams PH (1991) What to protect? Systematics and the agony of choice. Biological Conservation 55(3): 235–254. https://doi.org/10.1016/0006-3207(91)90030-D

Vanerelli AA, Miller AH, Comsa LC, Geneva AJ, Reynolds RG (2022) Mitochondrial genome of the critically Endangered silver boa (*Chilabothrus argentum*; Squamata: Boidae). Mitochondrial DNA Part B 7(6): 1180–1182. https://doi.org/10.1080/23802359.2022.2089067

Vidal N, Hedges SB (2005) The phylogeny of squamate reptiles (lizards, snakes, and amphisbaenians) inferred from nine nuclear protein-coding genes. Comptes Rendus Biologies 328(10–11): 1000–1008. https://doi.org/10.1016/j.crvi.2005.10.001

Vitt LJ, Caldwell JP (2013) Herpetology: An introductory biology of amphibians and reptiles. Academic Press, Cambridge, 35–82. https://doi.org/10.1016/B978-0-12-386919-7.00002-2

Wiens JJ, Hutter CR, Mulcahy DG, Noonan BP, Townsend TM, Sites Jr JW, Reeder TW (2012) Resolving the phylogeny of lizards and snakes (Squamata) with extensive sampling of genes and species. Biology Letters 8(6): 1043–1046. https://doi.org/10.1098/rsbl.2012.0703

Wüster W, Peppin L, Pook CE, Walker DE (2008) A nesting of vipers: phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). Molecular Phylogenetics and Evolution 49(2): 445–459. https://doi.org/10.1016/j.ympev.2008.08.019

Xiaokaiti X, Hashiguchi Y, Ota H, Kumazawa Y (2002) Evolution of the noncoding features of sea snake mitochondrial genomes within Elapidae. Genes 13(8): e1470. https://doi.org/10.3390/genes13081470

Yan J, Li H, Zhou K (2008) Evolution of the mitochondrial genome in snakes: Gene rearrangements and phylogenetic relationships. BMC Genomics 9(1): 1–7. https://doi.org/10.1186/1471-2164-9-569

Yao LZ, Gong YA, Tang XS (2020) Complete mitochondrial genome of the MacClelland's Coral Snake *Sinomicrurus macclellandi* (Reinhardt, 1844) (Serpentes: Elapidae). Mitochondrial DNA Part B 5(3): 2582–2584. https://doi.org/10.1080/23802359.2020.1781571

Yi CH, Park J, Sasai T, Kim HS, Kim JG, Kim MS, Cho IY, Kim IH (2019) Complete mitochondrial genome of the Ijima's Sea Snake (*Emydocephalus ijimae*) (Squamata, Elapidae). Mitochondrial DNA Part B 4(2): 2658–2659. https://doi.org/10.1080/23802359.2019.1641438

Zhang J, Yan C (2020) The mitochondrial genome of the *Hydrophis curtus* (Squamata: Elapidae). Mitochondrial DNA Part B 5(3): 3096–3097. https://doi.org/10.1080/23802359.2020.1800430

Zhang H, Liu Q, Lu C, Deng J, Huang X (2021) The first complete mitochondrial genome of Lachninae species and comparative genomics provide new insights into the evolution of gene rearrangement and the repeat region. Insects 12(1): 55. https://doi.org/10.3390/insects12010055

Zhu F, Liu Q, Zhong G, Xiao R, Fang M, Guo P (2016) Complete mitochondrial genome of *Sinovipera sichuanensis* (Reptilia: Squamata: Viperidae). Mitochondrial DNA Part A 27(5): 3666–3667. https://doi.org/10.3109/19401736.2015.1079846